10. The method of claim 1 wherein the measurement of P4H activity is via a ratio of P4H to proline.

11. The method of claim 1 wherein the DNA encoding is part of a combinatorial library.

REMARKS

The September 25, 2002 Office Action has rejected claims 1 through 21 under 35 U.S.C. § 112. In light of the amendments above and the arguments below, Applicants respectfully request reconsideration.

Applicants note that the invention of claims 1 through 21 was found to be free of prior art (See page 7 of current Office Action).

Typographical Error

Applicants have corrected a typographical error in claim 12. "Pch-1" should be phy-1.

§ 112 Rejections

Claims 1 through 21 are rejected under 35 U.S.C. § 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains to make and use the invention. Specifically, on page 4 of the Office Action, the Office Action states that

"the claimed invention encompasses any nematode" and further comments that "neither the specification nor the art of record teaches that any nematode would have the phenotype dpy or embryonic lethal when P4H gene is mutated in the nematode." While not agreeing with the Office Action's characterization of the specification, Applicants have now amended the claims so that *C. elegans* is claimed.

The Office Action then queries whether the dpy phenotype or embryonic lethal phenotype is specific to only the P4H gene. Applicants note that there are other genes in *C. elegans* that can be mutated to the dpy or embryonic lethal phenotype, but there are methods, known to one of skill in the art, for determining which gene is responsible for the phenotype. For example, one could grow the dpy mutations in a phy-1 background. This combination would be lethal and would only occur if the phy-1 mutation was in combination with the PH4 gene mutation. Applicants draw the Examiner's attention to the Friedman, et al. PNAS paper, referenced below and in the Office Action, as describing tests performed by crossing phy-1 mutants with dpy-18 mutants.

The Office Action then questions the enablement of "...chimeric nematode,", "P4H-gene modified nematode," and "a complementing P4H gene mutation." The Office

Action comments that the specification teaches what the chimeric nematode and P4H-gene modified nematode are but comments that the specification "does not teach as to how the nematodes will be produced." Applicants note that the Friedman, et al., [illegible], PNAS article cited by Applicants is incorporated by reference and forms a part of the present specification. Although this article was not available as a published document at the claimed priority date, the priority application was drafted from the text of the paper, and Applicants direct the Examiner to provisional application 60/154,267, beginning at page 3 of the specification. "Chimeric nematodes" and "gene modified nematodes" are both described.

The Office Action then queries whether a nematode that had a mutated P4H gene could be used to assay a compound that increased the activity of P4H. Applicants posit the situation where a mutation makes a defective protein that is somehow enhanced by the test composition.

The Office Action then questions whether a human P4H gene or P4H gene of any other organism can rescue the P4H activity in a dpy-18 or other mutant nematode. Applicants note that there is in vitro support for mixed species enzyme activity. Evidence in support of C. elegans P4H activity with the human PDI is shown [illegible] Veijola, et al. [illegible] which shows that C. elegans

prolyl 4-hydroxylase cDNA when expressed in the baculovirus system with the *C. elegans* PDI protein disulphide isomerase, a multifunctional polypeptide identical to the β subunit of P4H in the human PDI forms of "active prolyl 4-hydroxylase" (see Abstract). Prolyl 4-hydroxylase activity was assayed by a "method based on the decarboxylation of 2-oxo[1-14C]glutarate". (See page 7139, second column, third paragraph.)

This paper discusses multiple forms of PDI within the worm. In Table 1, prolyl 4-hydroxylase activity of Triton X-100 extracts of cells expressing human or *C. elegans* alpha subunits with the human PDI/beta subunit, *C. elegans* PDI beta, *C. elegans*-human or human *C. elegans* PDI beta subunit are disclosed. This table shows that that hybrid enzyme human alpha/*C. elegans* beta does have prolyl 4-hydroxylase activity.

The Office Action has rejected claims 1 through 21 under U.S.C. § 112, second paragraph as being indefinite.

Claim 1 has been rejected "because it is unclear as to what is encompassed by the phrase "a complemented prolyl-4 hydroxylase beta subunit." Applicants have amended this language to "a P4H β-subunit that complements an inactive P4H β-subunit."

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Claim [illegible] has been rejected on the ground of insufficient antecedent basis for the limitation "inhibitor." Claim [illegible] has been amended so that the inhibitor is now the "test compound."

Claim [illegible] is rejected as unclear. Applicants have amended the claim to include the phrase "the test chimeric nematode is a *C. elegans* and harbors a dpy-18 mutation."

Claim 12 is rejected on the ground of insufficient antecedent basis. Applicants have amended the claim to clarify that the "*Caenorhabditis elegans*" is meant.

Claim 17 is rejected on a similar rejection to claim 1. Applicants have made an identical amendment.

Claim 17 is rejected on the ground that the "test nematodes" in line [illegible] lacks antecedent basis. Claim 17 has been rewritten to focus on test chimeric *Caenorhabditis elegans* in both the 3rd and 6th lines.

Claims 1[illegible], [illegible], 1[illegible], 11, 1[illegible] and 14 are rejected on the limitation "the nematode" in line 1. These claims have all been cancelled as depending on independent claims.

<u>Entity Status</u>

Applicants wish to inform the U.S. Patent Office that they are eligible for small entity status.

Applicants have enclosed a Petition and Fee for Three Months Extension of Time. No other fees are believed necessary to enter this response. However, if any fees are necessary, please charge Deposit Account 17-0055.

Respectfully submitted,

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March 21, 2003

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants: Judith E. Kimble, et al.
Serial No.: [illegible]
Filed: September [illegible]
For: ASSAYS FOR MODULATORS OF PROLYL-4-HYDROXYLASE
Group Art Unit: [illegible]
Examiner: [illegible]

MARKED UP COPY OF THE CLAIMS

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1. (Amended) A method for evaluating a test compound's ability to modulate prolyl-4-hydroxylase (P4H), comprising the steps of:

(a) introducing a test compound into a test chimeric [nematode] *Caenorhabditis elegans*, a P4H-gene modified [nematode] *Caenorhabditis elegans*, or a wild-type [nematode] *Caenorhabditis elegans*, wherein the test chimeric [nematode] *Caenorhabditis elegans* [has a complemented prolyl-4-hydroxylase gene mutation] comprises a P4H gene that complements an endogenous P4H gene mutation, and

(b) observing the effect of the test compound on the prolyl 4-hydroxylase activity of the progeny of the test nematode, P4H-gene modified nematode or the wild-type nematode, wherein a dpy or embryonic lethal phenotype indicates prolyl-4-hydroxylase inhibition.

3. The method of claim 1, wherein the test compound is a chemical.

4. Amended The method of claim 1, wherein the [inhibitor] test compound is a protein or peptide.

4. The method of claim 1, wherein the introduction of the test compound into the plant-like nematode is a solution containing the test compound.

5. The method of claim 1, wherein the test compound is introduced into a wild-type nematode and the observation of dpy or embryonic lethal phenotype indicates nematode prolyl 4-hydroxylase inhibition.

6. The method of claim 1, wherein the test compound is introduced into a P4H-gene modified nematode and the observation of a dpy or embryonic lethal phenotype indicates P4H inhibition.

7. The method of claim 1, wherein the introduction of a test compound is into a test chimeric nematode and the observation of dpy or embryonic lethal phenotype indicates nematode prolyl 4-hydroxylase inhibition.

7. Amended. The method of claim 1, wherein the test chimeric nematode is a C. elegans and [is] harbors a dpy-1 mutation.

8. The method of claim 1, wherein the presence of a dpy phenotype indicates that the test compound modulates the P4H gene found on chromosome III.

12. Amended. A method for evaluating a test compound's ability to modulate prolyl 4-hydroxylase, comprising the step of:

(a) introducing a test compound into a [nematode] Caenorhabditis elegans comprising a dpy-10 or [pon-1] phy-1 mutation phenotype, and

(b) observing the effect of the test compound on the prolyl-4-hydroxylase activity of the progeny of the [test nematode] Caenorhabditis elegans, wherein the presence of the dpy-10 or phy-1 phenotype indicates an increased level of prolyl-4-hydroxylase activity.

17. The method of claim 1 wherein the test compound is part of a combinatorial chemical library.

18. The method of claim 12 wherein the test compound is part of a combinatorial chemical library.

17. Amended. A method for evaluating a test compound's ability to modulate P4H, comprising the steps of:

(a) introducing a test compound into a test chimeric [nematode] *Caenorhabditis elegans*, a P4H-gene modified [nematode] *Caenorhabditis elegans*, or a wild-type [nematode] *Caenorhabditis elegans*, wherein the test chimeric [nematode] *Caenorhabditis elegans* has a complemented P4H gene mutation, and

(b) measuring the level of P4H activity of the progeny of the test [nematode] *Caenorhabditis elegans*, P4H gene modified [nematode] *Caenorhabditis elegans* or wild-type [nematode] *Caenorhabditis elegans*, wherein a lower P4H activity compared to untested control [nematode] *Caenorhabditis elegans* indicates that the test compound is an inhibitor of P4H.

18. The method of claim 17 wherein the measurement of P4H activity is via a ratio of P4H to proline.

19. The method of claim 17 wherein the test compound is part of a combinatorial library.